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Influence of T-2 Mycotoxin on Host Resistance to Candida Albicans Infections in Mice[1,2]

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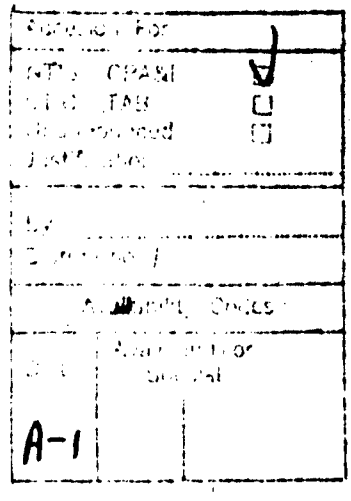

Mice infected with Candida albicans and then exposed to two or three doses of T-2 mycotoxin by the gastric route died earlier and in greater numbers than controls. The ability of livers, lungs, and spleens to clear and kill the organisms was compromised by exposing mice to multiple doses of toxin. A single dose of toxin given before or after Candida challenge did not increase the mortality. These studies add further information regarding biological effects of this mycotoxin, and shows that increased hazards are involved when this compound amplifies the pathogenesis of microorganisms such as C. albicans.

T-2 toxin, a trichothecene mycotoxin, is the principal toxic substance produced by several species of the common mold Fusarium. Fusarium species are important plant pathogens, causing a wilting of the host by physically blocking xylem vessels. In addition, the toxins produced by these fungi are thought to affect permeability of cell membranes and disrupt cell metabolism (1). Humans and domestic animals are also affected by T-2 toxin (2, 9). Ingestion causes hemorrhagic lesions, leucopenia, sepsis, and in sufficient concentrations, can cause alimentary toxic aleukia, a fatal disease first reported in Russia, attributed to consumption of over-wintered grain (11). Moldy corn containing this agent has been reported to cause disease in domestic animals (9). T-2 toxin has been implicated as a biological warfare agent, reportedly used in Southeast Asia and Afghanistan (8, 22).

Fusarium toxins are known to inhibit protein synthesis (16) and have a variety of cytotoxic properties (20, 23, 24). T-2 is an immunosuppressive agent (13, 14, 19), affecting macrophage functional activity (7, 15) as well as the capability of blood leukocytes to kill microorganisms such as Staphylococci effectively (24). Decreased resistance to mycobacterial infection in mice fed T-2 toxin indicates that this agent suppresses the cell-mediated immune system (12). Therefore, T-2 toxin injected in small quantities over an extended time, or as a single bolus rendered the animal more susceptible to microbial infections. Candida albicans, an opportunistic organism, is a common inhabitant of the oral cavity and intestinal tract. In an immunosuppressed individual or in one receiving broad-spectrum antibiotics, this organism can become highly pathogenic, infecting almost every organ and tissue of the body (18, 21). In candidiasis, as in most other infectious diseases, the major host defense mechanism is cell-mediated immunity. Our studies demonstrate the enhanced pathogenesis in mice of C. albicans after repeated oral doses of T-2 mycotoxin.

MATERIALS AND METHODS

Yeast. A culture of *C. albicans*, isolate M819, obtained from a human case of candidiasis (Dr. Howard W. Larsh, Missouri State Chest Hospital, Mt. Vernon, Mo) was used in all studies. The organisms were maintained on Sabouraud's dextrose agar slants incubated at 37°C. Viability studies on each fresh inoculum were done with a series of dilutions and plating on Sabouraud's dextrose agar incubated at 37°C for 48 h. Viability counts consistently ranged from 95% to 100%. Cells for inoculation were cultured in Sabouraud's dextrose broth enriched with 0.5% yeast extract at 37°C for 12 to 18 h with shaking. Hemocytometer counts were made and suitable inocula prepared by washing the cells three times and diluting with sterile physiological saline.

T-2 toxin. Toxin was purchased from Myco Labs, Inc., Chesterfield, Mo. The purity (> 98%) was determined by thin-layer chromatography and gas chromatography/mass spectrometry.

Animals. Twenty- to thirty-g, male Balb-c mice were obtained from Frederick Cancer Research Facility Animal Production Facility, Frederick, Md. Animals were fasted for 24 h before receiving toxin or carrier.

Titration of toxin for inoculation. T-2 toxin was dissolved in 50% propylene glycol:phosphate-buffered saline (PBS) at a concentration to give the required dose contained in 0.1 ml. Mice were dosed by gavage with a 20-ga animal-feeding-needle attached to a 1-ml syringe. The acute toxicity of T-2 by the oral route was determined by inoculating five groups of five mice each with toxin at 4, 6, 8, 10, and 12 ml per kg of body weight.

Titration of *C. albicans* for inoculation. Groups (size indicated in Table 1) of mice were first dosed with either propylene glycol carrier or T-2 toxin at 8 mg/kg then treated at 24 h with *C. albicans* inoculated i.v. at doses ranging from 1×10^3 to 1×10^6 contained in 0.5 ml. Deaths were recorded daily.

Multiple exposure to toxin. Fifty-five mice were inoculated i.v. with 1 x 10^5 C. albicans organisms and then divided into four groups, three test groups with 15 mice each, and one control group with five mice. Ten of the mice in one test group (Ia) received 0.1 ml of toxin (8 mg/kg) on day 3 and the five mice (Ib) received propylene glycol carrier. The same dose of toxin or propylene glycol was similarly administered to two additional groups on days 3 and 5 (IIa and IIb), and on days 3, 5, and 7 (IIIa and IIIb) after yeast administration. Ten control animals received only yeast.

Culture of C. albicans from organs. Four groups of mice (six per group) were inoculated with propylene glycol or one, two, or three doses of toxin spaced at 48-h intervals. The day after the last toxin exposure mice were injected i.v. with 1.5 x 10^5 C. albicans. Half the animals from each group was killed 1 h after C. albicans inoculation and the other half killed at 48 h. Lungs, livers, and spleens were removed and homogenized in a glass homogenizer (Belco Glass, Vineland, N.J.). Dilutions in PBS were plated onto Sabouraud's dextrose agar plates. Colonies of C. albicans were counted after 48-h of incubation at 37°C.

Blood clearance assays. Four groups of six mice each were inoculated with toxin or propylene glycol on the same schedule as described above and then inoculated with 1 x 10^5 organisms. Blood was collected from the retro-orbital plexus of each animal in heparinized capillary pipettes at 1 min, 60 min, 4 h, and 24 h. The samples were serially diluted in sterile saline and plated on Sabouraud's dextrose. Colonies of C. albicans were counted after 48 h incubation at 37°C.

Data analysis. Cumulative percent mortality was plotted against days after yeast exposure. Mean ± standard error were calculated where appropriate. The LD_{50} was calculated by probit analysis. Analysis of variance was performed with the SAS Statistical Package with Tukey's Studentized Range Test, $p < 0.01$.

Index of resistance = $(X_2 - X_1) - (Y_2 - Y_1)$ where:

X_1 = log CFU at 1 h from control mice;

X_2 = log CFU at 48 h from control mice;

Y_1 = log CFU 1 h from T-2-injected mice; and

Y_2 = log CFU 48 h from T-2-injected mice.

RESULTS

Titration of T-2 by the gastric route. Results of the toxin titration (data not shown) produced a calculated LD_{50} of 11 mg per kg of body weight. The group receiving the highest dose (8 mg/kg) with no deaths was selected for subsequent experiments.

Titration of C. albicans. Table 1 shows the lethality of our C. albicans strain for mice at 14 and 21 days. The calculated LD_{50} was 1.4×10^5 organisms at 14 days and 5.2×10^5 organisms at 21 days. In another series of experiments, mice were given C. albicans intraperitoneally (data not shown). The intraperitoneal LD_{50} was 2.5×10^8 organisms per mouse. Since the intravenous dose was much lower, subsequent studies were carried out with a dose of 1×10^5 organisms injected intravenously per mouse and terminated at 14 days.

Effect of T-2 toxin on a lethal C. albicans infection. Table 1 also shows that there was no significant difference between control animals and those given one sublethal (8 mg/kg) dose of T-2. Similar results (not shown) were obtained when mice received C. albicans i.p. and were then challenged with a single oral dose of T-2.

Effects of multiple toxin exposures on mice previously infected with C. albicans. Results in Figure 1 show that three exposures to T-2 had a significant effect on lethality in mice carrying the yeast load. Thirty percent were dead by day 6 and 100% were dead by day 10 after yeast infection. With two doses of mycotoxin, 20% were dead by day 6, 40% by day 10, and 60% by day 14. In both the C. albicans-only control group and the group receiving one dose of T-2 plus C. albicans, 20% of the mice were dead by day 14. There were no deaths (data not shown) in mice exposed to two doses of toxin without previous exposure to C. albicans; and 27% were dead by 10 days in the group receiving three doses.

Culture studies on organ homogenates. Viable C. albicans recovered from organs at each time point are shown in Table 2. Toxin impaired the organs from animals receiving two or three doses to resist yeast infection. Large numbers of viable yeast remained in lungs of mice in the two- or three-exposure group. In the group exposed to three doses of toxin, the number of viable organisms increased from 39,000 to 147,000 over 48 h. In all animals receiving one dose or no toxin, organisms were completely cleared from the lungs in 48 h. Figure 2 shows the index of resistance for livers and spleens of animals receiving multiple doses of toxin. Two or three exposures to toxin caused a decrease in resistance (increase in susceptibility) in both organs, with the spleen showing the most significant decrease.

Effect of T-2 toxin on the clearance of C. albicans from the blood. Results of the blood clearance studies are shown in Table 3. There was >94% clearance in all groups by 1 min after an intravenous bolus of 1×10^5 organisms. Analysis of variance between the control group and the three experimental groups indicate there was no difference between the dose groups in their ability to clear the organisms.

DISCUSSION

Many chemical and toxic agents are known to influence the susceptibility of animals to opportunistic organisms, including bacteria, viruses, and yeast. It is thought that the primary mode of action is by impairment of the immune system, although other mechanisms, such as alterations in metabolism, are possible (3). Previous studies on the immunosuppressive activity of T-2 toxin have shown impairment of antibody synthesis and rejection of skin grafts (19), reduction of T and B cell mitogenesis (13), compromised in vitro alveolar macrophage function (7) and leukopenia, lymphopenia, and lymphoid depletion (5). The results of our studies demonstrate a pronounced effect of T-2 toxin given by the oral route on the pathogenesis of C. albicans. Fromentin et al. (6) demonstrated similar effects with the mycotoxin diacetoxyscirpenol and Kanai and Ko..do (12) demonstrated an effect of chronic oral administration of T-2 on the pathogenesis of mycobacteria. In our experiments a single oral dose of T-2 was insufficient to give any alterations in the death rate due to C. albicans or its clearance from spleen, liver, or lungs. However, two or three oral doses of T-2 significantly decreased the ability of lungs and spleen to clear the organism.

Blood clearance of C. albicans was rapid, with >94% gone by 1 min. Virtually all the organisms were cleared by 4 h. It is evident that T-2 did not affect significantly the clearance of C. albicans from blood even after three sequential boli. The mechanism to clear microorganisms from the blood was therefore unaffected by T-2 toxin. Although metabolic inactivation of T-2 decreases its toxicity (4), significant toxicity remains (4). It appears from our experiments that the mouse can detoxify a single bolus without any effect

on the animal's ability to handle opportunistic infections. However, with repeated exposure, the level of toxin as well as its metabolites accumulate to levels that are toxic in vivo for lymphocytes.

The results of these studies present evidence that multiple exposure to T-2 toxin has a significant synergistic effect on the pathogenesis of the opportunistic organism C. albicans. They substantiate previous reports showing T-2 to be an immunosuppressive agent and the importance of possible contamination of food products by T-2 on the lowered resistance of animals to opportunistic infections.

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TABLE 1. Lethality of *C. albicans* in Mice With and Without T-2 Toxin[a]

C. albicans Dose	Lethality			
	Control		Plus T-2 Toxin	
	14 days	21 days	14 days	21 days
1×10^3	0/3[b]	0/8	0/6	0/6
1×10^4	1/13	2/13	2/6	2/6
1×10^5	3/13	8/13	3/6	3/6
1×10^6	13/13	13/13	5/5	5/5
Control	0/5	0/5	1/5	1/5
LD_{50}	1.4×10^5	5.2×10^4	4.6×10^4	4.6×10^4

[a] T-2 toxin in propylene glycol or propylene glycol only given by gavage 24 h before inoculation with *C. albicans*. Dose was 8 mg/kg in 0.1 ml.

[b] Number dead/number injected.

TABLE 2. Mean viable C. albicans recovered from organs of control mice and T-2 toxin-injected mice 1 and 48 h after challenge with 1.5×10^5 yeast cells injected i.v.

Group	T-2 Toxin[a]	Propylene glycol[b]	Count after 1 h[c]			Count after 48 h		
			Lungs	Livers	Spleens	Lungs	Liver	Spleens
I	1	0	34,000 ± 1,155	55,000 ± 5,685	3,413 ± 237	0	7,766 ± 636	2,660 ± 98
II	2	0	TNTC[d]	38,333 ± 4,096	3,013 ± 106	27,667 ± 5,207	14,833 ± 766	2,626 ± 43
III	3	0	39,000 ± 4,619	27,667 ± 1,667	450 ± 87	147,660 ± 333	36,000 ± 2,309	10,000 ± 611
IV	0	1	23,667 ± 1,667	55,667 ± 6,839	[e]	0	18,466 ± 1,556	2,800 ± 702
V	0	2	26,667 ± 882	57,333 ± 333	4,466 ± 240	0	6,800 ± 251	2,520 ± 248
VI	0	3	7,667 ± 882	37,000 ± 3,786	3,733 ± 533	0	7,500 ± 650	2,586 ± 466
VII[f]	0	0	40,667 ± 12,197	68,667 ± 2,906	5,933 ± 240	0	7,066 ± 88	3,153 ± 261

[a] Number of injections, T-2 toxin, 8 mg/kg; 0.1 ml/mouse, in propylene glycol given by gavage. Gr. I, given one dose toxin, 1 day before Candida challenge; Gr. II given two doses toxin, 3 days and 1 day before challenge; Gr. III given three doses, 5, 3, and 1 day before challenge with C. albicans.

[b] Control animals, injected with propylene glycol carrier alone, same schedule as (a).

[c] Average of three mice (± S.E.).

[d] TNTC means too numerous to count.

[e] Value missing due to technical error. Means of groups V, VI, and VII (controls) used to calculate index of resistance (Fig. 2).

[f] Control animals, injected only with C. albicans.

TABLE 3. Blood clearance of viable *C. albicans*[a,b]

Group	Counts			
	1 MIN	60 MIN	4 H	24 H
I[c]	2287 ± 596	412 ± 82	210 ± 71	31 ± 11
II	4837 ± 1721	900 ± 97	206 ± 48	31 ± 18
III[d]	4200 ± 637	1200 ± 281	131 ± 18	[e]
IV	5587 ± 1817	693 ± 101	180 ± 36	12 ± 7

[a] 1×10^5 organisms inoculated i.v.; six animals per group.

[b] Blood (0.4 ml) collected from retro-orbital plexus. Results expressed as number of viable organisms ± standard error per total blood volume (1.5 ml).

[c] Group I: one dose of T-2; Group II: two doses of T-2; Group III: three doses of T-2; Group IV: saline control.

[d] N = 2. Four animals died before inoculation with *C. albicans*.

[e] Animals dead by 24 h.

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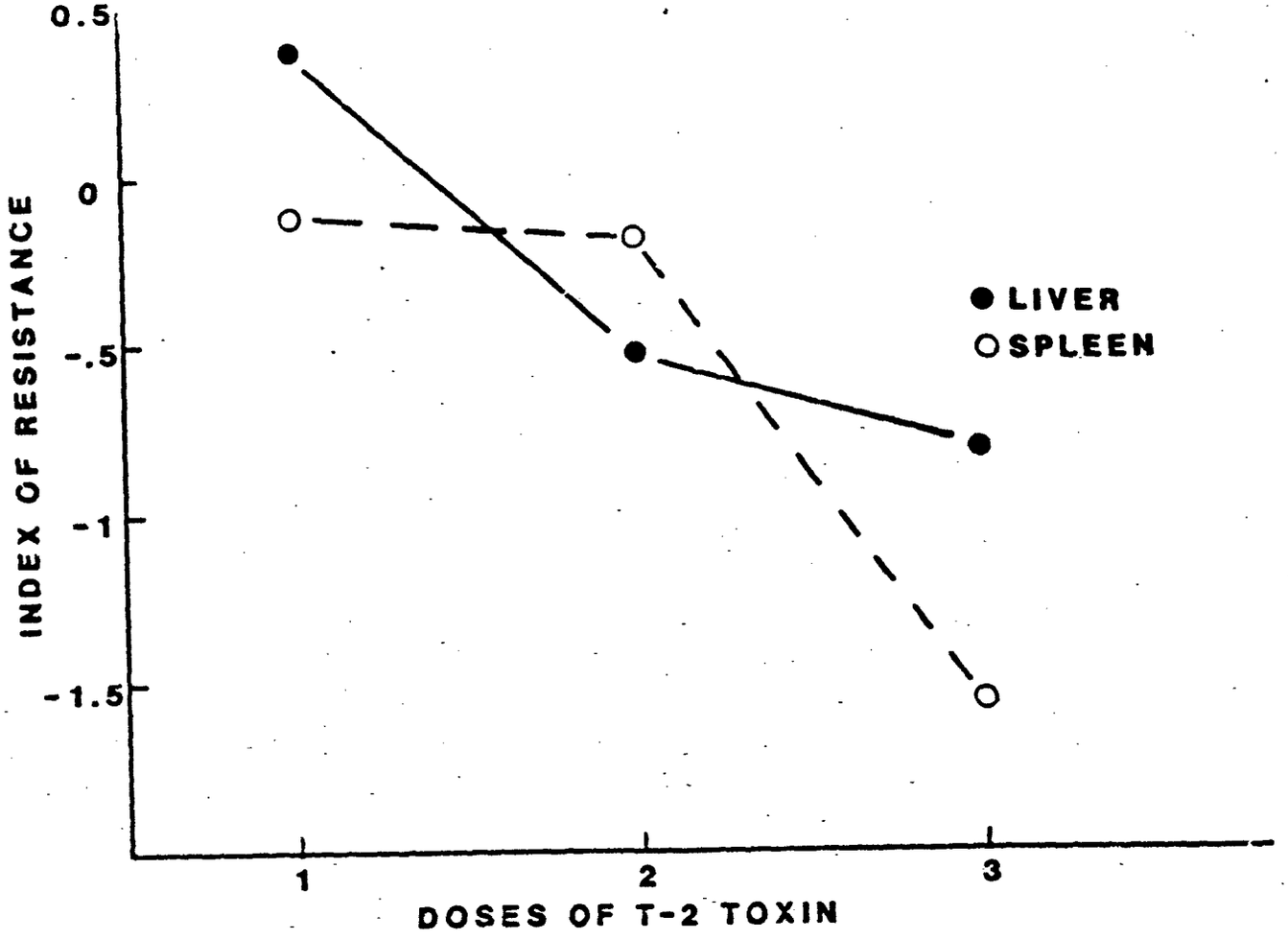
[e] Animals dead by 24 h.

FIG. 1. Lethal effects of multiple injections of T-2 toxin in mice infected with Candida albicans. Mice received 1 x 10^5 cells of C. albicans, i.v.

FIG. 2. Index of resistance to C. albicans injection in livers and spleens of mice pretreated with one, two, or three exposures to T-2 mycotoxin.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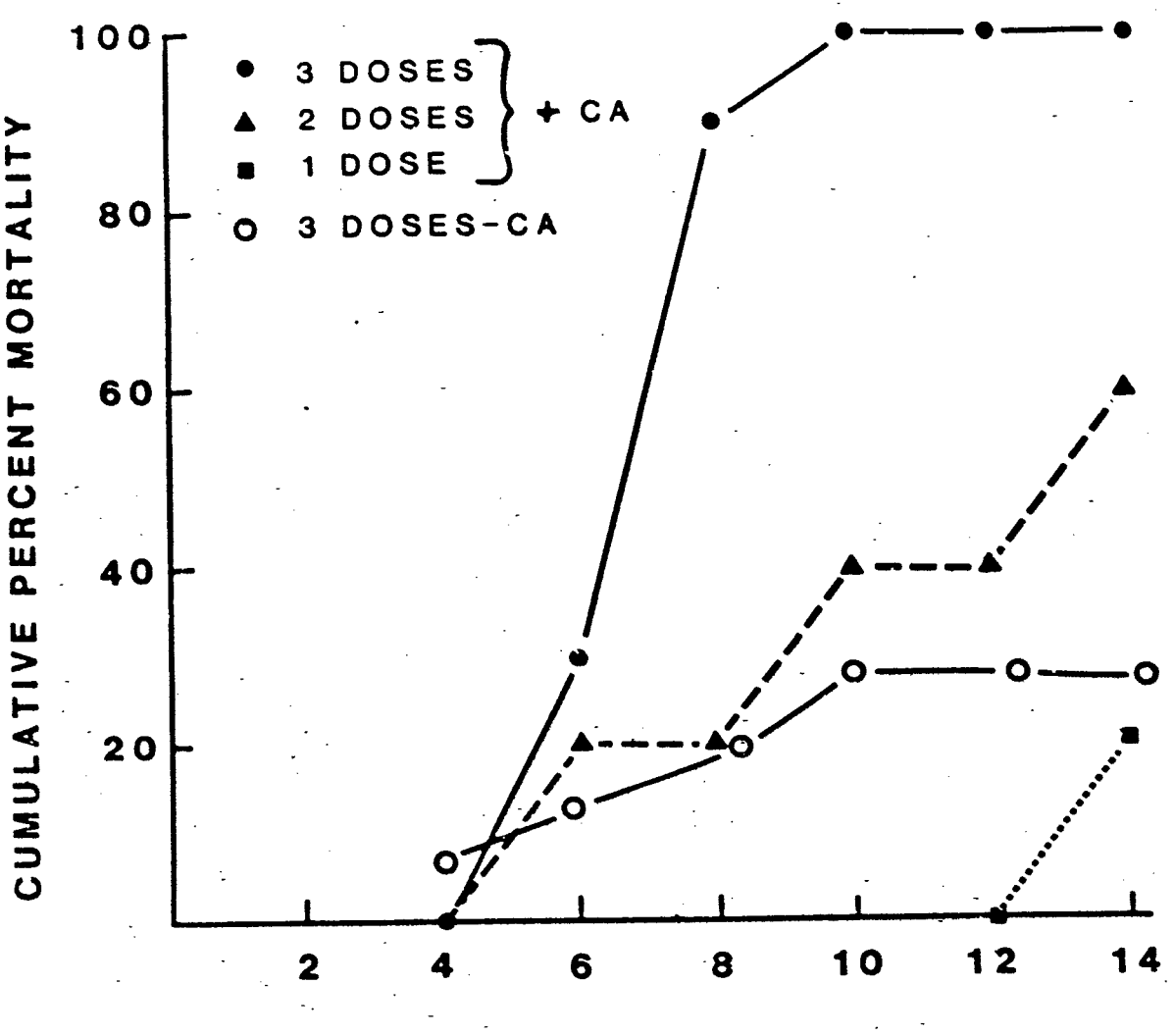
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